Bol. San. Veg. Plagas, 25: 311-320, 1999

The pool of Coccinellids (Coleoptera: Coccinellidae) to control Coccids (Homoptera: Coccoidea) in Portuguese citrus groves

A. MAGRO[1] & J. L. HEMPTINNE[2]

Integrated Pest Management programmes in Portugal pay main attention to coccids. The study of coccinellids for the biological control of those pests was undertaken.

This paper aimed firstly to determine which coccidophagous ladybirds are more likely to control coccids by examining Dominance, Constancy and Persistence along the year. A second goal was to shed some light on the feeding regime of several species of the Scymnini tribe.

Eight coccidophagous ladybirds have been observed to have a strong presence in Portuguese citrus groves. They correspond to species regularly considered as important for the biological control of coccids in other Mediterranean countries.

Five species of the *Scymnus* genus are also very important in the sampled orchards. Though usually considered as aphidophagous, their abundance is correlated to the appearance of coccinellids which are predators of coccids.

A first large scale phenology of the most important ladybird beetles in citrus orchards is provided which can help to schedule chemical interventions.

Key words: Coccinellids, coccids, citrus, biological control, *Scymnus* spp.

INTRODUCTION

Integrated pest management research programmes have been developed in Portuguese citrus groves since 1990. Main attention is paid to *Planococcus citri* (Risso), *Saissetia oleae* (Olivier), and *Lepidosaphes beckii* (Newman) that are considered as key pests in continental Portugal (CARVALHO *et al.*, in press).

Biological control is an important issue in the above mentioned programmes. The study of the coccinellids present in citrus groves and their contribution for the biological control of coccids was undertaken for these predators have proved often successful in the fight against the referred phytophagous (HODEK, 1970, COPPEL & MERTINS, 1977, IPERTI, 1987, MAJERUS, 1994, DIXON, *et al.*, 1997).

A large scale survey of citrus orchards in Portugal revealed the richness of communities of coccidophagous ladybirds (MAGRO, *et al.*, submitted). There were also many members of the Scymnini tribe the feeding preferences of which are hardly known.

The tendency in biological control is to neglect the vast potential of indigenous predators. Frequently they are often regarded as inefficient and practitioners tend to adopt classical biological control and to rely on well known exotic species that are subse-

[1] Ecole Nationale Supérieure Agronomique de Toulouse, Av. de l'Agrobiopole, Auzeville-Tolosane, BP 107-F31326 Castanet - Tolosan Cedex, France.

[2] Ecole Nationale de Formation Agronomique, BP 87-F31326 Castanet-Tolosan Cedex, France.

quently introduced. Such an approach is clearly illustrated in the context of attempts to control aphids. *Coccinella septempunctata* L. has been purposely released in the USA against cereal aphids despite the presence of diversified guilds of indigenous ladybirds (SCHAEFER, *et al., 1987).* On the contrary, in Europe, C *septempunctata* has a reputation of inefficiency (CRAWLEY, 1992) and there is a move towards releasing *Harmonia axyridis* Pallas. Similarly, the coccidophagous *Cryptolaemus montrouzieri* Mulsant is now established all around the world and, in Portugal, *Nephus reunioni* Fürsch was carelessly tested and released in the vicinity of Lisbon. It is now regularly extending its range throughout the country (MAGRO, *et al.,* submitted).

The side effects of these introductions that often follow an hit-and-miss (KAREIVA, 1996) approach are now questioned (HOWARTH, 1991, TOWNSEND, 1991, MILLS, 1994, CLOUT, 1995, ELLIOTT, *et al.,* 1996, SIMBERLOFF & STILING, 1996). The best solution to avoid such possible effects is to use the word «efficient» with an extreme prudence and to improve the knowledge on indigenous predators.

This paper prolongs previous works on biological control of citrus coccids (MAGRO, 1997, MAGRO, *et al.,* in press). It aims firstly to determine which coccidophagous species are more likely to control coccids by examining dominance, constancy and persistence of species in different communities. A second goal is to shed some light on the feeding regime of several species of the Scymnini tribe.

MATERIALS AND METHODS

Samples of adult coccinellids were collected in citrus groves from Algarve, Setúbal and Santarém, the three main citrus regions in Portugal (Table 1).

Ladybirds were collected following the «Beating method», recommended by IOBC (AMARO & BAGGIOLINI, 1982). 100 branches uniformly distributed in each orchard were selected and beaten with a stick. In order to standardise the sampling method, each branch was stricken three times. The insects so dislodged fell in a j ar attached at the bottom of a tray in a form of a funnel. This method mainly yielded adult ladybirds but few larval instars. Orchards were visited one or two times a month as indicated in Table 1.

Coccinellids were identified with keys published by CHAZEAU, *et al.* (1974), GOURREAU (1974), RAIMUNDO & ALVES (1986), FÜRSCH (1987 *a, b*) and RAIMUNDO (1992). Most identifications were achieved by considering external characters but extraction and observation of genitals was needed for about 1100 individuals.

ANALYSIS OF RESULTS

Dominance, Constancy and Persistence

The values of Dominance *(D)* and Constancy *(C)* were calculated for the guilds of coccid predators and those with unknown food preferences in citrus in each grove and year:

$$D = \frac{n_i}{N} \times 100 \text{ and } C = \frac{p_i}{p} \times 100$$

Table 1. - **Period, periodicity of sampling, number of sampled groves (NG) and total number of collected samples (CS) per region.**

Region	NG	Period	Periodicity	CS
Algarve	12	August 1993 - July 1994	Monthly	131
Setúbal	2	13.3.91 - 21.2.94	Every week till the end of October 1991 and every two weeks afterwards	168
Santarém	1	24.5.91 - 23.2.94	Every week till the end of October 1991 and every two weeks afterwards	74

where n_i is the number of captured individuals in species i, N is the total number of captured individuals, p_i is the number of samples where the species i was present and p the total number of collected samples.

Mean and extreme values of those two parameters where calculated for each of the three years of captures in the groves of Setúbal and Santarém and for the total of the 12 groves in Algarve. Species were then classified according to the following intervals:

	Dominance
Dominant	[25% - 100%]
Sub-dominant	[12% - 25%]
Accessory	[1% - 12%]
Vestigial	[0.01% - 1%]

	Constancy
Constant	[40% -100%]
Sub-constant	[20% - 40%]
Incidental	[1% - 20%]

Persistence *(P)*, which measures the number of years a species is present in the samples, was calculated for the groves of Setúbal and Santarém.

The importance of the presence of different coccinellids in citrus was then compared on the basis of the values of *D*, *C* and P.

Periods of activity

The results of captures from all the groves and years were considered. For each month and species, the mean number of captured individuals per month was calculated. The percentage of activity *(A)* was calculated:

$$A = \frac{Mv_{ij}}{TMv_i} \times 100$$

where Mv_{ij} is the mean value of captures of species i in the month j and TMv_i the sum of all the monthly mean values for species i (along the 12 months of a year). Six classes of activity were defined: 0% (absence of activity); [0.1% – 4.9%]; [5% – 9.9%]; [10% – 19.9%]; [20% – 39.9%]; [40% – 100%].

Covariation between species

Covariation was calculated from the activity periods (see 2.). Spearman's rank coefficients were used because they best evaluate similarities between species based on abundance data (LEGENDRE & LEGENDRE, 1984 and LUDWIG & REYNOLDS, 1988). The software «Stecol» (LUDWIG & REYNOLDS, 1988) was chosen for the analysis.

In order to identify the groups of species closer in terms of adult population dynamics in citrus, an hierarchical and agglomerative clustering based on the results of Spearman's rank coefficient was performed. Distance between objects was computed using Lance and Williams linear combinatorial equation with parameters corresponding to Ward's method. Clustering was done with the software «Progiciel R. Analyse multidimensionnelle, analyse spatiale» (LEGENDRE & VAUDOR, 1991). The results of the cluster analysis were sumarised in a dendrogram.

Sampling for immature stages of *Scymnus* spp and *Nephus* spp.

From Spring to Summer 1995 samples of fruits, leaves and young branches of citrus infested with *S. oleae, L. beckii,* various mealybugs, *A. floccosus* and different aphids were collected. The samples were brought back to laboratory and carefully searched for eggs and larval stages of *Scymnus* spp. and *Nephus* spp.

RESULTS

19112 individuals from 38 species were captured and seven species were only represented by one individual (Table 2).

Table 2 - **Total number of species of Coccinellids captured in citrus groves from the regions of Setúbal, Santarém and Algarve, Dominance values (Dv - %) and food preferences (Fp). N = total number of individuals. 1 = Diaspididae, 2 = Coccidae, 3 = Pseudococcidae, 4 = *Icerya purchasi*, 5 = aphids, 6 = mites, 7 = white flies, 8 = plants, 9 = fungus, 10 = unknown food preferences in citrus orchards.**

TAXA	Dv	Fp	TAXA	Dv	F
Chilocorini	**10.81**		**Noviini**	**3.62**	
Chilocorus bipustulatus L.	8.30	1, 2	*Rodolia cardinalis* (Mulsant)	3.62	4
Exochomus nigromaculatus (Goeze)	1.28	5	**Platynaspini**	**0.02**	
E. quadripustulatus L.	1.22	1, 2, 5	*Platynaspis luteorubra* Goeze	0.02	1
Coccidulini	**9.09**		**Scymnini**	**72.38**	
Lindorus lophantae (Blaisdell)	5.66	1, 2	*Clitosthetus arcuatus (Rossi)*	5.27	7
Rhizobius chrysomeloides (Herbst)	3.39	1	*Cryptolaemus montrouzieri* Mulsant	2.50	3
R. litura Fabricius	0.04	1	*Nephus bisignatus* Boheman	0.02	10
Coccinellini	**4.02**		*Nephus includens* (Kirsch)	5.13	3
Adalia bipuncatata (L.)	0.17	5	*Nephus reunioni* Fürsch	1.57	3
A. decempunctata (L.)	1.56	5	*Nephus binotatus* Brisout	0.01	10
Coccinella septempunctata L.	0.15	5	*Nephus ulbrichi* Fürsch	0.03	10
Oenopia conglobata (L.)	0.14	5	*Nephus hickei* Fürsch	0.01	10
O. doublieri (Mulsant)	0.01	5	*Nephus fuerschi* Plaza	0.01	10
O. lyncea (Oliv.)	0.01	5	*Scymnus mediterraneus* Khnzorian	18.07	10
Propylea quatuordecimpunctata (L.)	1.96	5	*Scymnus auritus* (Thunberg)	0.01	lo
Psyl lobora vigintiduopunctata (L.)	0.03	9	*Scymnus subvillosus* (Goeze)	4.53	10
Tytthasp¡s sedecimpunctata (L.)	0.01	9	*Scymnus suturalis* Thunberg	0.04	10
Epilachnini	**0.01**		*Scymnus apetzi* Mulsant	1.91	10
Subcoccinella vigintiquatuorpunctata L.	0.01	8	*Scymnus interruptus* (Goeze)	22.23	10
Hippodamiini	**0.05**		*Scymnus levaillanti* Mulsant	1.26	10
Hippodamia variegata (Goeze)	0.05	5	*Scymnus rufipes* (Fabricius)	0.03	10
Hyperaspini	**0.01**		*Stethoruspunctillum* (Weise)	9.77	6
Hyperaspis reppensis Herbst	0.01	10			
N				**19112**	

A few individuals of the species *Nephus peyerimhoffi* Sicard, claimed by Raimundo (1992) as being present in Portugal, might be among those identified as *N. includens* which would raise the number of species to 39. We were not able to distinguish these two species due to the unclear descriptions of the genitalia of *N. peyerimhoffi* provided by Raimundo (1992) and Fürsch (1987b).

The Scymnini tribe is the richer and more dominant one with 18 species corresponding to 72% of captured individuals.

Table 3 *(a* and *b)* shows the results of Dominance, Constancy and Persistence for the coccid predators and those with unknown food preferences in each region. Note that Dominance values are always calculated against total values that is for all coccinellid species. Dominance and Constancy are variable for each species either in the same year in different groves or in different years in the same grove. However some patterns emerge; the ladybirds can be sorted out in five groups:

Group I - Dominant and Constant species:

C. bipustulatus, R. chrysomeloides, N. includens, S. mediterraneus, S. subvillosus, S. interruptus and *S. punctillum.*

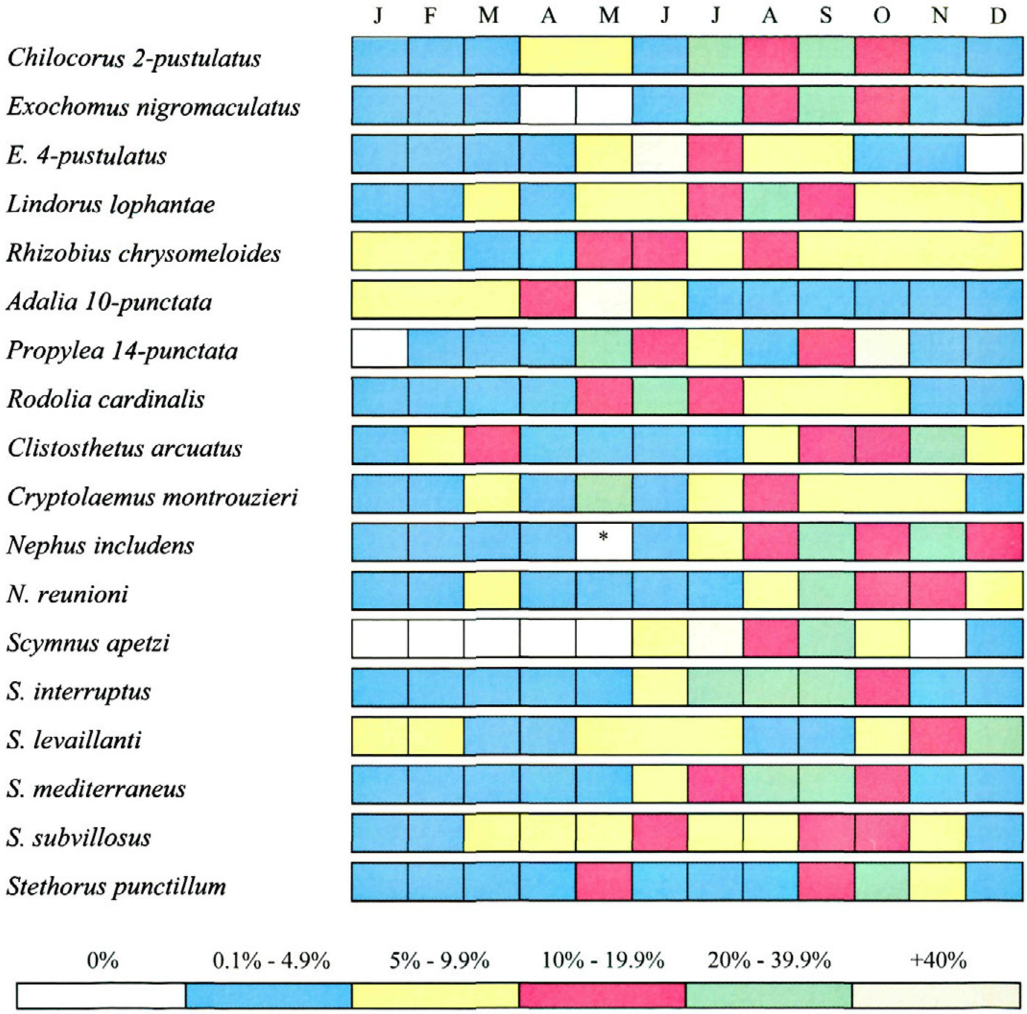

Fig. 1. - Periods of activity (monthly) of some species of coccinellids in citrus in Portugal.
* absence of data for this month.

Group II - Sub-dominant and Constant species:

L. lophantae, R. cardinalis, C. montrouzieri and *S. apetzi.*

Group III - Accessory and Constant species:

E. quadripustulatus, N. reunioni and *S. levaillanti.*

Group IV - Accessory and Incidental species:

N. ulbrichi.

Group V - Vestigial and Incidental species:

R. litura, H. reppensis, P. luteorubra, N. bisignatus, N. binotatus, N. hiekei, N. fuerschi, S. auritus, S. suturalis and *S. rufipes.*

Persistence follows the same decreasing tendency.

The periods of activity of species in groups I to III as well as some well known and abundantly represented coccinellids from other guilds *(A. decempunctata, P. qua-*

a)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1		-0.077	0.049	0.341	-0.704	0.175	-0.175	-0.393	-0.387	0.489	0.123	0.112	-0.361	-0.091	-0.228	-0.172	0.063	0.193
2			0.27	0.343	0.053	-0.105	0.427	0.809	0.775	0.221	-0.315	-0.277	0.071	0.322	0.235	0.266	0.811	0.049
3				0.621	0.474	0.634	0.771	0.401	0.51	0.546	0.438	-0.011	0.692	0.858	-0.249	0.753	0.182	0.701
4			*		0.023	0.588	0.704	0.469	0.239	0.382	0.354	0.407	0.226	0.55	-0.261	0.399	0.291	0.399
5	*					0.466	0.624	0.431	0.533	0.071	0.469	0.187	0.851	0.667	0.15	0.656	-0.046	0.462
6			*	*			0.643	0.036	0.081	0.536	0.748	0.564	0.657	0.804	-0.336	0.734	-0.042	0.895
7			**	*	*	*		0.673	0.565	0.41	0.58	0.343	0.788	0.825	0.105	0.72	0.322	0.58
8		**					*		0.817	0.301	0.136	0.118	0.372	0.455	0.56	0.255	0.745	0.073
9		**	*		*		*	**		0.1	-0.119	-0.337	0.463	0.611	0.09	0.579	0.474	0.218
10			*			*					0.578	0.237	0.346	0.49	0.119	0.259	0.508	0.725
11						**	*			*		0.683	0.668	0.503	0.091	0.364	-0.056	0.678
12						*					*		0.157	0.158	0.165	0.046	-0.091	0.308
13			*		**	*	**				*			0.825	0.056	0.802	0.011	0.694
14			**	*	*	**	**		*				**		-0.2	0.93	0.168	0.832
15								*								-0.319	0.224	-0.291
16			**		*	**	**		*				**	**			0.042	0.769
17		**						**		*								0.182
18			*			**	*			**	*		*	**		**		

1- *A. 10-punctata*
2- *C. arcuatus*
3- *C. 2-pustulatus*
4- *C. montrouzieri*
5- *E. nigromaculatus*
6- *E. 4-pustulatus*
7- *L. lophantae*
8- *N. includens*
9- *N. reunioni*
10- *P. 14-punctata*
11- *R. cardinalis*
12- *R. chrysomeloides*
13- *S. apetzi*
14- *S. interruptus*
15- *S. levaillanti*
16- *S. mediterraneus*
17- *S. punctillum*
18- *S. subvillosus*

b)

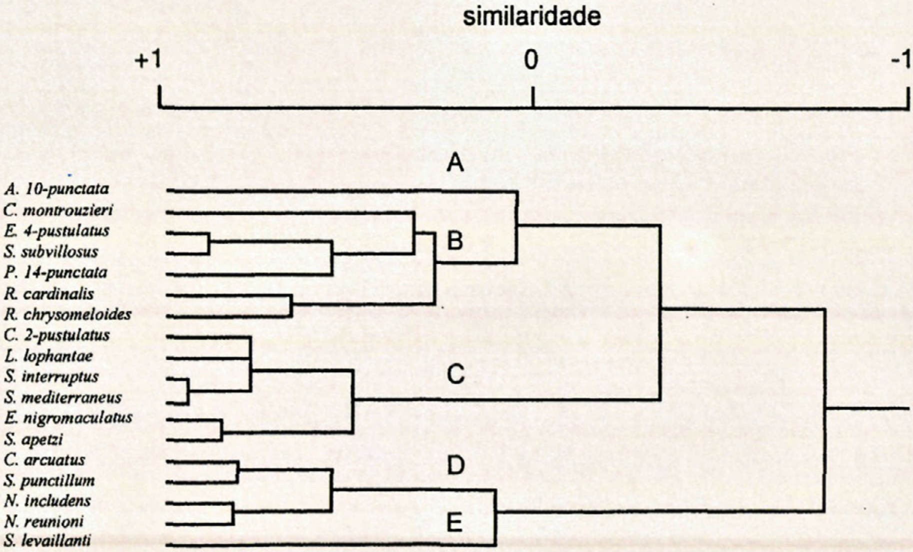

Fig. 2. - Covariance between coccinellid species in terms of activity periods in citrus. a) Covariation matrix. Upper part presents values of Spearman's rank coefficient and lower part the significatif covariation (* - α=0,05; ** - α=0,01). b) Dendrogam based on the degree of positive covariation between species (Ward's method). A to E - main detected groups.

tuordecimpunctata, E. nigromaculatus and *C. arcuatus)* are presented in Figure 1. Summer time is the period where the activity is higher but we can see that the majority of the coccinellids can be present all over the year.

The matrix of covariation between the activity periods and the corresponding dendrogram lead to five groups of species (Figure 2, *a* and *b)*. Group A is an assemblage of species having their main activity in Spring and group E in Autumn. The three remaning display intermediate periods of activity.

The analysis of Figures 1 and 2 can give us some cues on *Scymnus* spp. possible food preferences: *S. interruptus* and *S. mediterraneus* belong to group C and were mainly caught in Summer. Their activity is highly correlated with that of *C. bipustulatus, L. lophantae* and *E. quadripustulatus*. It is therefore suggested that *S. interruptus* and *S. mediterraneus* are probably predators of coccids and diaspidids. *S. apetzi* could be included in the same group but is neverthless distinctive by a long period during which it does not turn up, in the samples. *S. subvillosus* behaves very similarly to *E. quadripustulatus* which can either feed on coccids, diaspidids or aphids. *S. levaillanti* comes very late being more close to the mealybug predators *N. reunioni* and *N. includens*, the mite predator *S. punctillum* or the white-flies predator *C. arcuatus*.

A. decempuncata and *P. quatuordecimpunctata* are two true aphid predators. There is no species that display a clear correlation with their activity (Fig. 1). The two highest values of Spearman's rank coefficient arose with the two *Exochomus* that have a wide range of prey and feed on aphids. Although their fecundity is higher when feed on coccids they can lay eggs on an aphid diet (RADWAN & LÖVEI, 1982).

In the plant material brought to laboratory for the control of immature stages of *Scymnus* and *Nephus* species, we were never able to find eggs or larvae but for *S. interruptus* and *S. subvillosus* on aphid colonies at spring time. Larvae of *L. lophantae* and *C. bipustulatus* were though present on coccids and diaspidids, *A. decempunctata* and *P. quatuordecimpunctata* on aphids and *C. arcuatus* on white-flies.

DISCUSSION AND CONCLUSIONS

The tribes Chilocorini and Coccidulini are better represented in the samples from citrus orchards than the Coccinellini (Table 2). The Scymnini tribe is however dominant in terms of richness and abundance but the ecology of all these species remain unclear.

Dominance and Constancy values (Table 3 *a* and *b*) were highly variable. This is not exclusive to coccinellids but is a consequence of the interaction between species of any community and their relationship with a fluctuating environment (BEGON, et al., 1996). Variability does not hide a pattern as the species are distributed in five groups in terms of their dominance, constancy and persistence in the communities. That is the dominant species were always the same even if the hierarchy within the group was variable in time and space. The same applies to the other categories. This situation was also observed for aphidophagous coccinellid guilds in different habitats (HEMPTINNE, 1989) and for parasitoid guilds (EHLER, 1994, MILLS, 1994b). There are hypothesis to explain these changing hierachies but they need to be experimentally tested. In a perspective of biological control, the impact of species ranking on pest populations remains an important research topic (Ehler, 1994).

Ladybirds in groups I, II and III have the strongest presence in citrus orchards.They represent a potential pool of species for biological control of citrus coccids in Portugal. They are regularly considered in such a way by KEHAT & GREENBERG (1970), LONGO & BENFATTO (1987), CLIMENT (1990a and b) and MARI, *et al.* (1991). *Scymnus* spp. are sometimes mentioned for citrus orchards in other countries but nobody grant them much importance.

The species of the genus *Scymnus* are usually considered as aphidophagous (e.g.

Table 3. - **Species of Coccinellids captured in citrus groves from the regions of Setúbal (Vidais and Várzea), Santarém (C. Cadaval) and Algarve (12 groves).** a) **Distribution in classes of Dominance: V (vestigial) = [0.01%-1%[, Ac (accesory) = [1% - 12%[, Sd (sub-dominant) = [12% - 25%[, D (dominant) = [25% - 100%].** b) **Values of Persistence (P) and distribution in clases of Constancy: In (incidental) = [1% - 20%[, Sb (sub-constant) = [20% - 40%[, C (constant) = [40% - 100%]. * species present in only one sample. ■ = mean value and ├┤ = variation interval (minimum and maximum values).**

a) Species	Vidais (V Ac Sd D)	Várzea (V Ac Sd D)	C. Cadaval (V Ac Sd D)	Algarve (V Ac Sd D)
Chilocorus 2-pustulatus				
E. 4-pustulatus				
Lindorus lophantae				
Rhizobius chrysomeloides				
R. litura		*		*
Hyperaspis reppensis	*			
Rodolia cardinalis				
Platynaspis luteorubra	*			
Cryptolaemus montrouzieri				
Nephus 2-signatus				
N. includens				
N. reunioni				
N. 2-notatus				*
N. ulbrichi	*		*	
N. hiekei				
N. fuerschi				
Scymnus mediterraneus				
S. auritus	*			
S. subvillosus				
S. suturalis				
S. apetzi				
S. interruptus				
S. levaillanti				
S. rufipes				*

b) Species	Vidais (In Sc C)	Vidais *P*	Várzea (In Sc C)	Várzea *P*	C.Cadaval (In Sc C)	C.Cadaval *P*	Algarve (In Sc C)	Algarve *np*
Chilocorus 2-pustulatus		3				3		11
E. 4-pustulatus		3				3		6
Lindorus lophantae		3		2		3		10
Rhizobius chrysomeloides		3		3		3		8
R. litura		2	*	1		1	*	1
Hyperaspis reppensis	*	1						
Rodolia cardinalis		3		3		3		12
Platynaspis luteorubra	*	1						3
Cryptolaemus montrouzieri		2				3		6
Nephus 2-signatus								2
N. includens								12
N. reunioni		3		3				
N. 2-notatus							*	1
N. ulbrichi	*	1			*	1		1
N. hiekei								2
N. fuerschi								1
Scymnus mediterraneus		3		3		3		12
S. auritus	*	1						
S. subvillosus		3		3		3		12
S. suturalis		2		1				
S. apetzi		3		1		2		7
S. interruptus		3		3		3		12
S. levaillanti		3		3		3		6
S. rufipes		1		0			*	1

HODEK, 1973, IPERTI, 1983, CLIMENT, 1990b). On the contrary, in our samples the presence of adults is correlated to the appearance of coccinellids predators of other pests. They are not correlated with the periods of activity of two true aphid predators, *A. decempunctata* and *P. quatuordecimpunctata*. These results suggest that several *Scymnus* increase the pool of coccidophagous species. Experiments have to be undertaken to clarify that point. At the moment it is not possible to firmly rule out aphidophagy among the Scymnini of this study. They could be breeding on aphids outside the orchards and move in later in the season in response to large quantity of honeydew excreted by coccids and white-flies. HAGEN, *et al.* (1970) and EVANS (1994) have demonstrated the powerful attraction of honeydew for several predators.

C. montrouzieri is the most commonly used predator in biological control against *P. citri*. In the late 25 years *N. reunioni* was introduced in several countries and used in inondative releases sometimes in conjuntion with *C. montrouzieri* (ALEXANDRAKIS, 1984, PROTA, *et al.*, 1984, LONGO & RUSSO, 1986). However, this strategy of control is not supported by studies on the dynamics or the relative efficiency of each species. The present study points out that as expected these two species are syncronized with their host and therefore opens the question of the existence of competition between the two predators.

Finally, this work provides a first large scale phenology of the most important ladybird beetles in citrus orchards. lt is a valuable data in the context of an integrated pest management because pesticides applications can be scheduled to minimize harmful effects on the beneficial fauna.

ACKNOWLEDGEMENTS

This paper was written while the first author was on a postdoctoral fellowship of the Fundação para a Ciência e Tecnologia - Portugal - (Programme Praxis XXI). The research was supported by a PhD grant from the same programme.

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(Recepción: 9 abril 1999)
(Aceptación: 13 agosto 1999)